

The Agar Gel-Diffusion Technique as a Method of Tracing Mosquito Antigens in the Water Bug *Anisops breddini* (Notonectidae)

Anisops, in common with other notonectids, is predatory and feeds mainly on aquatic insects of various kinds (BROOKS¹). In Asia, species of *Anisops* have been classified as important predators on mosquito larvae (LAIRD²). In the laboratory, they can be raised on an exclusive diet of mosquito larvae (HALE³).

However, direct evidence of feeding on mosquito larvae under field conditions has not been obtained. It was felt that this evidence could be obtained if antiserum prepared against mosquito larvae reacted positively with *Anisops*.

The antigens used in the gel-diffusion tests were saline extracts of fourth instar *Culex pipiens fatigans* larvae and of *Anisops*. The antiserum used was obtained by immunizing rabbits with saline extract of fourth instar *Culex pipiens fatigans* larvae. Each rabbit received approximately 1500–2000 larvae in divided doses with Freund's adjuvant, given subcutaneously. The gel-diffusion plates were prepared with 1% Ion-agar No. 2 (Oxo) in distilled water, containing 0.01% Na merthiolate as bacteriostatic agent. The holes were cut with Feinberg's cutters (Shandon), and the reaction was allowed to take place at room temperature in a moist chamber.

Three batches of *Anisops*, obtained at different times from the field, were tested against the anti-larval serum. In the first two batches the results were negative. In the third batch, precipitate lines common to the fourth instar *Culex* larvae were observed (Figure 1). The controls in this experiment consisted of *Anisops* from the same batch, which were starved for 48 h. The positive result with only one batch is not unexpected because continuous feeding on mosquito larvae is unlikely in the field.

Subsequently, a laboratory experiment was set up in which *Anisops* were supplied with fourth instar *Culex* larvae. They were first starved for 48 h to get rid of mosquito antigens before the experiment was started. The antigens were then prepared from *Anisops* which had fed on fourth instar *Culex* larvae after different time intervals, of 1, 6, 12, and 24 h. The controls consisted of starved *Anisops* from the same batch. It is seen in Figure 2 that some of the larval antigens can be clearly traced in *Anisops* for upto 6 h after feeding. The precipitate lines becoming indistinct after this period.

Zusammenfassung. Mit der Agar-Gel-Diffusionsmethode konnte das Mückenantigen in der sich von Mückenlarven ernährenden Wasserwanze *Anisops breddini* (Notonectidae) bis zu 6 h nach der Nahrungsaufnahme nachgewiesen werden.

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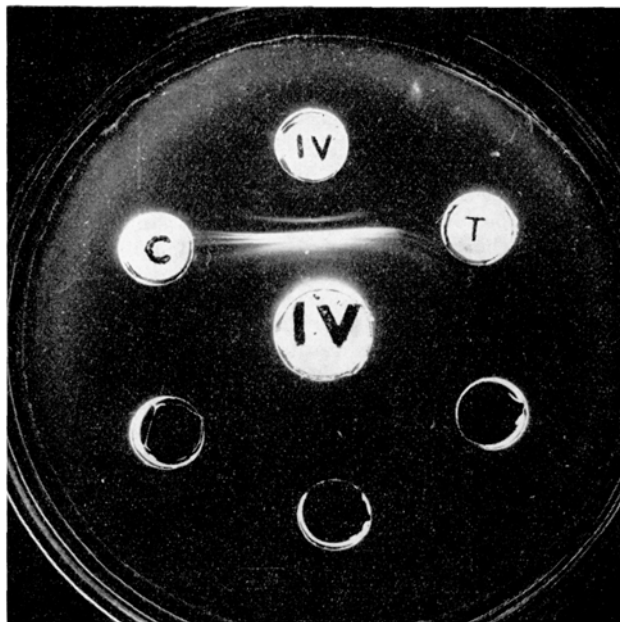


Fig. 1. IV Central well = IV instar *Culex* antiserum. IV Peripheral well = IV instar *Culex* antigen. C = Anisops antigen control. T = Anisops antigen test.

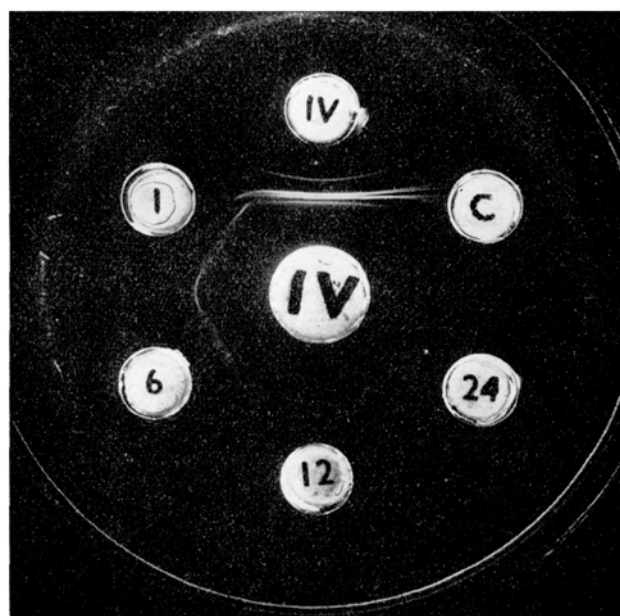


Fig. 2. IV Central well = IV instar *Culex* antiserum. IV Peripheral well = IV instar *Culex* antigen. 1 = 1 h Anisops antigen. 6 = 6 h Anisops antigen. 12 = 12 h Anisops antigen. 24 = 24 h Anisops antigen. C = Anisops antigen control.

¹ G. T. BROOKS, Kansas Univ. Sci. Bull. 34, 301 (1951).

² M. LAIRD, Bull. R. Soc. N.Z. 6, 213 (1956).

³ H. M. HALE, Rec. S. Aust. Mus. 2, 397 (1923).